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Mechanisms of Mitochondrial Defects in Gulf War Syndrome

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14. ABSTRACT
This is the Annual Summary of funding for this proposal. Human Protection Approval was received on 10/13/2009. This report covers the first 9 months of work for the study. Patients are being recruited and laboratory procedures are being established for the study according to the Statement of Work.

15. SUBJECT TERMS
Gulf War Syndrome, mitochondria, fatigue, myalgias

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Introduction:
Gulf War syndrome (GWS) is associated with increased incidences of amyotrophic lateral sclerosis, pain syndromes, muscle complaints that include fatigue and myalgias, as well as other neurological symptoms. Approximately 100,000 individuals have medical complaints consistent with GWS. Clinical manifestations are similar to those identified in Chronic Fatigue Syndrome (CFS). Mitochondrial defects are identified pathologically, metabolically, and genetically in some patients with CFS. GWS has significant evidence for mitochondrial dysfunction with abnormalities in exercise physiology, abnormalities in mitochondrial morphology, biochemical defects in mitochondrial function, abnormalities in free radical generation affecting mitochondrial integrity, gene expression in genes affecting mitochondrial function, and mtDNA mutations (inherited, somatic, and sporadic during embryogenesis). Gene expression abnormalities in CFS show abnormalities in genes that are related to mitochondrial function. Hence, investigation of mitochondrial dysfunction in GWS is a priority.

Body:
Quarter 1 of research: Human Protection Approval obtained 10/13/2009. Requires data collection on significant number of subjects for appropriate analysis. This is the first quarter of funding for this proposal. Human Protection Approval received on 10/13/2009. We are in the earliest phases of study implementation: (a) establishing funding distribution, (b) establishing recruitment mechanisms (physician letter, internet, Veteran's Administration Hospital). Two patients have been recruited for study. Approximately 5 additional individuals are under review (receiving clinical records, clinical appointment scheduling, coordination of laboratory testing. All laboratory procedures necessary for this grant are in place.

Brief Summary of SOW Tasks:
Task 1: (Specific Aim 1) Months 1-24 (Note: HRP Approval Granted 10/14/2010. We are in month 9 of the study)
Fifty veterans with GWS who have fatigue and myalgias will be identified through the Atlanta Veterans Administration Medical Center and via website postings. Clinical records are requested and reviewed by the P. I. to confirm diagnosis of GWS. If the records are consistent with inclusion criteria, an appointment is made for clinical examination by the P.I., blood draw and skin biopsy. Modified criteria for chronic fatigue syndrome and fibromyalgia will be used as guides for patient inclusion criteria, thus allowing comparison of the GWS patient data with CFS/fibromyalgia patient data. Based on published percentages of study groups showing evidence for mitochondrial defects, we predict that approximately 60-70% of GWS patients will harbor mitochondrial defects.

Progress:
1. Five individuals are in the process of being recruited and entered into study. Records are being received and reviewed so that inclusion/exclusion criteria can be assessed.
2. Internet recruitment has been established
3. Notification/recruitment via patients at the Atlanta Veterans Administration Hospital has been established. We are notifying other Veteran Administration Hospitals so that they are aware of our study.

Task 2: (Specific Aim 2) Months 6-36 (Note: HRP Approval Granted 10/14/2010. We are in month 9 of the study)
Characterize mitochondrial cellular energetics in GWS patients relative to age and gender matched controls using the following approaches: (1) high resolution respirometry of intact cells [EBV transformed lymphocytes, cultured fibroblasts], (2) quantitative analysis of individual mitochondrial proteins (denatured, Western blot), (3) analysis of intact OXPHOS enzyme

complexes and supercomplexes (non-denatured, Blue Native and Clear Native gels), (4) in gel enzyme activity assessment of intact OXPHOS enzyme complexes and supercomplexes (Clear Native gel, in-gel activity measurements), (5) mtDNA copy number quantitation to assess for defects in regulating mtDNA replication, and (6) cellular coenzyme Q10 quantitation (endogenous synthesis is impaired in certain types of mitochondrial dysfunction).

Progress:

1. All laboratory analyses are established in the laboratory for use in the study. The status of each area of testing is outlined below.
    a. High Resolution Respirometry
        i. Fibroblast High Resolution Respirometry: 5%-95% reference intervals are validated for the parameters required for assessment of mitochondrial function. Reference ranges are established for the following parameters: uncoupling ratio, net routine flux control ratio, respiratory control ratio, leak flux control ratio, phosphorylation respiratory control ratio.
        ii. EBV transformed cell lines: We are in the process of establishing appropriate reference ranges for the same parameters.
    b. Western blot (denatured oxidative phosphorylation subunit) Quantitative analysis of individual mitochondrial proteins. The technique has been established and validated for muscle. Establishing reference intervals for fibroblasts is underway.
    c. Blue Native and Clear Native Analyses (NON-denatured analysis of supercomplex formation and monomeric oxidative phosphorylation enzyme assembly). These approaches assess supercomplex formation and monomeric oxidative phosphorylation enzyme assembly. The process is well validated for skeletal muscle. We are refining the techniques for assessment of fibroblast oxidative phosphorylation supercomplex and monomeric enzyme assembly.
    d. Clear Native Oxidative Phosphorylation Enzyme activity: This technique assesses activity of individual oxidative phosphorylation enzymes. Intact oxidative phosphorylation enzymes are isolated by gel electrophoresis and the activity assessed (as isolated enzymes).
    e. mtDNA copy number analysis: This technique is well validated for muscle. We are establishing reference ranges for fibroblast cell lines.
    f. Cellular Coenzyme Q10 quantitation for fibroblasts: This testing is established for skeletal muscle. The reference intervals are being established for fibroblasts.

Task 3: (Specific Aim 3) Months 6-36 (Note: HRP Approval Granted 10/14/2010. We are in month 9 of the study)

Assess the mitochondrial DNA (mtDNA) from each patient with GWS for mtDNA mutations by whole genome sequencing of leukocyte and skin cell mtDNA. Based on the findings from Specific Aim II, selected nuclear coded OXPHOS genes will be sequenced to assess for mutations that increase susceptibility to GWS.

Progress:

1. We have changed our laboratory approaches from capillary sequencing (Sanger sequencing) to Next Generation sequencing.
2. Samples will be banked so that all can be run more efficiently by Next Generation Sequencing approaches.

Key Research Accomplishments:

None to date. Study is in an early phase. Most data analysis for the Gulf War Syndrome patients will likely occur during the last 3-4 months of the study. Comparison of the Gulf War Syndrome

data with appropriate normal controls and disease groups is essential for interpretation of the data. We are analyzing data from patients with known pathogenic mutations affecting oxidative phosphorylation as well as patients with chronic fatigue/fibromyalgia diagnoses. Comparison of data from these groups with the Gulf War Syndrome patients is important.

Reportable Outcomes:
None to date. Requires data collection on significant number of subjects for appropriate analysis. Most data analysis for the Gulf War Syndrome patients will likely occur during the last 3-4 months of the study.

Conclusion:
None to date. Most data analysis for the Gulf War Syndrome patients will likely occur during the last 3-4 months of the study.

References:
NONE

Appendices
NONE